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US 4022911 A

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(54) Method of disinfection

(57) A method for disinfecting bacteriologically contaminated surfaces comprises the application to said surfaces of a composition comprising a synergistic mixture of (a) a quaternary ammonium compound and (b) a chlorinated methyl substituted phenol, in (c) at least one aqueous mono-hydric alcohol and, optionally, at least one poly-hydric alcohol; wherein the ratio of (a) to (b) is between 4:1 and 1:1.5. The compositions may further include sodium or potassium salts of ethylenediamine tetraacetic acid and are preferably produced as concentrates, for dilution with water when required.

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METHOD OF DISINFECTION

The invention relates to a method of disinfecting surfaces particularly surfaces contaminated with bacteria.

The prior art has long provided a wide variety of compounds capable, either singly or in combination with each other, of reducing microbial contamination although each class of compounds tends to have its own drawbacks. Examples of such antimicrobial compounds include cationic quaternary ammonium salts (eg di-long chain alkyl di-short chain alkyl ammonium chlorides); phenol and phenolic compounds, such as coal tar disinfectants (eg cresols and xylenols), bisphenols (eg triclosan), neutral oils of coal tar fractions (eg lower alkyl substituted naphthalenes) and phenol derivatives such as halogenated and/or alkylated phenols (eg 4-chlorophenol, 2-bromophenol etc); halogens either alone or as tinctures or complexes (eg alcoholic iodine, chlorine releasing compounds, povidone iodine complexes etc); biguanidines (eg chlorhexidine); aldehydes (eg glutaraldehyde) and alcohols (eg ethanol, isopropanol etc). All of the above groups of antimicrobial compounds have disadvantages of one kind or another, either because of gaps in their spectrum of activity, cost, safety, impracticality for formulation etc.

Combinations of actives are also well known particularly combinations of biguanidines with other antibacterials (eg chlorhexidine and cetrimide). Many of these combinations still suffer from the disadvantages of the parent compounds particularly with respect to limited

spectrum of activity etc.

US 4022911 (Goldhaft et al) discloses a combination containing, as the major component, formaldehyde with lesser amounts of (a) a quaternary ammonium compound and (b) a phenol or a derivative thereof. The major component (formaldehyde) is not only readily deactivated by the presence of proteins (ie such as blood) but is possibly carcinogenic and is thus of limited use as a household or human disinfectant.

EP 308210 (Beauchamp and Rogers) discloses a combination of (a) an antiseptic/anaesthetic including phenolics, (b) a quaternary ammonium compound and (c) antiseptic iodine salts or complexes, but activity is only demonstrated versus viral infections and skin disorders eg psoriasis or eczema.

US 4098877 (Ball et al) discloses a combination of (a) a phenolic bacteriostat (specifically triclosan) and (b) a long chain alkyl, phenoxyalkyl substituted quaternary bacteriocide (specifically dodecyl dimethyl (2-phenoxyethyl) ammonium bromide), though this teaches combinations in the range 4:1 to 6:1 phenolic to quaternary ammonium compound making them quite expensive.

Japanese unexamined applications JP 51-109911A (Sadaaki Nishimura) and JP 54-107519A (Kyoritsu Yukikogyo) both disclose alleged synergistic combinations of para-chloro-meta xylenol (PCMX) and quaternary ammonium salts for which there is only substantiated synergistic activity versus fungi. It is not always true that activity versus fungi

indicates activity versus bacteria. Certainly high fungicidal activity is no guarantee of high bacteriocidal activity.

We have now found that certain combinations of quaternary ammonium salts and chlorinated methyl substituted phenols exhibit synergistic antibacterial activity. This is surprising as quaternary ammonium salts and phenolic derivatives are normally considered to be incompatible. The synergistic effect is useful not only because it allows less of each active to be used in formulated products (reducing expense) but also because antibacterial activity is found at greater than normal dilutions, allowing a greater margin of safety in cases of over dilution.

According to this invention there is provided a method for disinfecting bacteriologically contaminated surfaces which comprises the application to said surfaces of a composition comprising a synergistic mixture of (a) a quaternary ammonium compound and (b) a chlorinated methyl substituted phenol, in (c) at least one aqueous mono-hydric alcohol and, optionally, at least one poly-hydric alcohol; wherein the ratio of the quaternary ammonium compound to the chlorinated phenol is between 4:1 and 1:1.5.

In another aspect of the invention there are provided compositions for use in the method comprising a synergistic mixture of (a) a quaternary ammonium compound and (b) a chlorinated methyl substituted phenol, in (c) at least one aqueous mono-hydric alcohol and, optionally, at least one poly-hydric alcohol; wherein the ratio of the quaternary

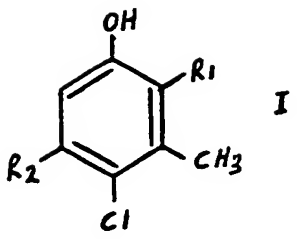
ammonium compound to the chlorinated phenol is between 4:1 and 1:1.5.

The synergistic activity of these compositions is particularly demonstrable versus gram negative bacteria for example Proteus, Escherichia, Klebsiella, Shigella, and Salmonella species.

Quaternary ammonium compounds may be considered as being organically substituted ammonium compounds of the formula $[R_1R_2R_3R_4N]^+X^-$ in which each R group is a substituted or unsubstituted alkyl, aryl, alkaryl, aralkyl or alkenyl group. Alternatively three of the four R groups may be combined in a pyridine ring. The sum of the carbon atoms in the four R groups is more than 10 and at least one of the R groups has a chain length in the range C_8 to C_{18}. X is a small anion.

Examples of commonly used quaternary ammonium compounds are 1-hexadecylpyridinium chloride (cetylpyridinium chloride); benzyldimethyl-2-{2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy}ethylammonium chloride (benzethonium chloride); mixtures of alkyldimethylbenzylammonium chlorides (benzalkonium chloride); mixtures of dodecyl-, tetradecyl- and hexadecyl-trimethylammonium bromides (cetrimide); mixtures of alkaryltrimethylammonium chlorides; or mixtures thereof.

Chlorinated methyl substituted phenols have the following general structure (I)



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where R_1 is chlorine or hydrogen, and

R_2 is methyl or hydrogen.

Examples include 4-chloro-3-methylphenol (PCMC), 4-chloro-3,5-dimethylphenol (PCMX) and 2,4-dichloro-3,5-dimethylphenol (DCMX); or mixtures thereof.

Suitable mono-hydric alcohols are the lower alkyl C_{1-4} alcohols (eg methanol, ethanol, propan-2-ol,n-butanol), or mixtures thereof.

Suitable poly-hydric alcohols are the polyethylene glycols of average molecular weight 200 to 1000 (particularly 200 to 600), propylene glycol, ethylene glycol or mixtures thereof.

It is well known that antibacterial activity may be increased versus certain bacterial species (eg Pseudomonas species) by the inclusion of a chelating agent such as ethylenediamine tetraacetic acid (EDTA) or its salts. Accordingly ethylenediamine tetraacetic acid may optionally be included in the compositions particularly as the di or tetra sodium or potassium salts.

Perfumes and colouring agents well known in the art may also be included for aesthetic considerations.

At use dilutions the compositions contain (a) from 0.02 to 0.2% w/v quaternary ammonium compounds, (b) from 0.01 to

0.15% w/v chlorinated methyl substituted phenols, (c) from 0.1 to 5% w/v total mono-hydric and poly-hydric alcohols, (d) from 0 to 0.10% w/v sodium or potassium salts of ethylenediamine tetraacetic acid, (e) colourings and perfumes as required, (f) water as balance.

For convenience the compositions may be produced in concentrated form for subsequent dilution with water to the use dilutions given above. The concentrates are most conveniently formulated such that they may be diluted from 1:10 to 1:100 to achieve use dilution.

The amount and proportion of mono-hydric and poly-hydric alcohols required in these concentrated formulations will be dependent on the natures and amounts of the quaternary ammonium compounds and the chlorinated methyl substituted phenols. It will be appreciated that when no poly-hydric alcohols are used the minimum concentration of the mono-hydric alcohols required will be higher than when poly-hydric alcohols are included.

Bactericidal evaluation was carried out according to a modification of the European Suspension Test (Council of Europe, Test method for the antimicrobial activity of disinfectants in food hygiene, Strasbourg 1987). The method is outlined below.

Second generation bacterial cultures are produced by growth on appropriate agar slopes. These are washed from the slopes in buffer and filtered before being adjusted to a concentration of approximately 10^8 cells/ml.

The disinfectants are diluted to twice the

concentration at which they are to be tested, in standard hard water.

1ml of bacterial culture is added to 4ml of bovine albumin solution (2% w/v), at 20°C, and the two are shaken for two minutes. 5ml of double strength disinfectant are added and the mixture is shaken at 20°C for five minutes. Samples are taken, neutralised, and the number of surviving organisms are enumerated using standard microbiological techniques.

Simultaneously with the above a control test is also carried out using hard water as the test solution.

The numbers of organisms surving five minutes contact with the disinfectant and with the water are used to calculate the Microbiological Effect (ME) of the disinfectant by the following formula

ME = Log NoC - Log NoT

where Log NoC = Log(10) number of organisms recovered from the control mixture

Log NoT = Log(10) number of organisms recovered from the disinfectant mixture.

When no organisms are recovered after contact with the disinfectant, the number of survivors is assumed to be less than the detection limit of the counting technique (normally 100 organisms per ml). Log NoT is therefore expressed as "less than" this value, so the Microbiological Effect of the agent is expressed as "greater than" (>).

Tests are repeated on at least three separate days and the median Microbiological Effect (Median ME) is used as the

final result.

The invention is illustrated by the following Examples.

Examples 1-4

A range of formulations were prepared to the following formula

	% (w/v)
Gloquat C *	see Table 1
4-Chloro-3,5-dimethylphenol (PCMX)	see Table 1
Propan-2-ol	10.0
Propylene glycol	25.0
Perfume **	0.25
Softened water	to 100%

*Gloquat C (ABM Chemicals, UK) is a mixture of alkaryltrimethylammonium chlorides.

**Felton "Antiseptic Fragrance"87.1619 (The Independent Fragrance Company, UK).

Formulations were prepared as follows:

1. stir Gloquat C in softened water
2. add propan-2-ol and stir well
3. add PCMX, stir well
4. add propylene glycol and stir until the PCMX is dissolved
5. add perfume and stir
6. make up to volume with softened water and stir well.

The formulations were tested using the modified European Suspension Test method described above. Final test concentrations were 1% and the test organism was Proteus mirabilis

Table 1 gives test data for the compositions of Examples 1 to 4 together with that for a comparative example and two controls (percentages are w/v in the concentrate).

TABLE 1

Example	Gloquat C	PCMX	Median M E
1	4%	2.5%	>6.0
2	4%	2.0%	>7.7
3	4%	1.5%	4.7
4	4%	1.0%	4.2
Comparative	4%	0.5%	0.7
Control	4%	0%	0.1
Control	0%	2.5%*	0

* The formulation was produced as a 0.05% PCMX solution in water, as 2.5% PCMX solutions cannot be produced without the presence of a quaternary ammonium compound. During microbial testing this is directly comparable to Example 1 as both will contain 0.025% w/v PCMX as test solutions.

The Microbiological Effect values in Table 1 clearly show that in Examples 1 to 4 Gloquat C and 4-chloro-3,5-dimethylphenol have synergistic antibacterial activity, with the mixtures having much higher activity than either compound alone. The synergy is particularly marked at ratios of Gloquat C to 4-chloro-3,5-dimethylphenol of between 4:1 and 4:2.5.

Examples 5-7

The compositions of Examples 1 to 4 were varied by replacing the 4-chloro-3,5-dimethylphenol with 4-chloro-3-methylphenol (PCMC) as shown in Table 2. Bactericidal

tests were carried out as described for Examples 1 to 4.

Table 2 gives test data for the compositions of Examples 5 to 7 together with that for two comparative examples and two controls (percentages are w/v in the concentrate).

TABLE 2

Example	Gloquat C	PCMC	Median M E
5	4%	2.5%	4.4
6	4%	2.0%	4.5
7	4%	1.5%	2.3
Comparative	4%	1.0%	0.2
Comparative	4%	0.5%	0.2
Control	4%	0%	0.1
Control	0%	2.5% *	0.1

* For explanation see footnote to Table 1

Synergy is demonstrated between Gloquat C and 4-chloro-3-methylphenol at ratios of between 4:1.5 and 4:2.5.

Examples 8-10

The compositions of Examples 1 to 4 were varied by replacing the Gloquat C with benzethonium chloride (BEC) as shown in Table 3. Bacteriocidal tests were carried out as described for Examples 1 to 4.

Table 3 gives test data for the compositions of Examples 8 to 10 together with that for a comparative example plus two controls (percentages are w/v in the concentrate).

TABLE 3

Example	BEC	PCMX	Median M E
8	4%	2.5%	>7.5
9	4%	2.0%	>6.3
10	4%	1.0%	5.3
Comparative	4%	0.5%	4.5
Control	4%	0%	4.0
Control	0%	2.5% *	0

* For explanation see footnote to Table 1.

Synergistic antibacterial activity is demonstrated between benzethonium chloride and 4-chloro-3,5-dimethylphenol at ratios of between 4:1 and 4:2.5.

Examples 11-14

The compositions of Examples 1 to 4 were varied by replacing the Gloquat C with benzalkonium chloride and replacing the 4-chloro-3,5-dimethylphenol with 4-chloro-3-methylphenol (PCMC) as shown in Table 4. Bacteriocidal tests were carried out as described for Examples 1 to 4.

Table 4 gives test data for the compositions of Examples 11 to 14 together with that for two comparative examples and two controls (percentages are w/v in the concentrates).

TABLE 4

Example	BKC	PCMC	Median M E
11	4%	2.5%	>6.0
12	4%	2.0%	>6.0
13	4%	1.5%	>5.7
14	4%	1.25%	>6.5
Comparative	4%	1.0%	4.2
Comparative	4%	0.5%	4.1
Control	4%	0%	4.3
Control	0%	2.5% *	0.1

* For explanation see footnote to Table 1.

Synergistic antibacterial activity is demonstrated between benzalkonium chloride and 4-chloro-3-methylphenol at ratios of between 4:1.25 and 4:2.5.

Example 15

The composition of Example 1 was varied by replacing the Gloquat C with benzalkonium chloride (BKC) as shown in Table 5. Bacteriocidal tests were carried out as described for Examples 1 to 4.

Table 5 gives test data for the composition of Example 15 together with that for two controls (percentages are w/v in the concentrate).

TABLE 5

Example	BKC	PCMX	Median M E
15	2%	3%	4.1
Control	2%	0%	0
Control	0%	3%*	0

* For explanation see footnote to Table 1.

Synergistic antibacterial activity is demonstrated between benzalkonium chloride and 4-chloro-3,5-dimethylphenol at a ratio of 1:1.5.

Examples 16 and 17

The composition of Example 2 was varied by replacing the Gloquat C with benzalkonium chloride (BKC) as shown in Table 6. Disodium ethylenediamine tetraacetic acid was included in Example 16 also as shown in Table 6. Bacteriocidal tests were carried out as described for Examples 1 to 4.

Table 6 gives test data for the compositions of Examples 16 and 17 together with that for two controls (percentages are w/v in the concentrates).

TABLE 6

Example	BKC	PCMX	Disodium EDTA	Median M E
16	4%	2%	0.75%	>6.2
17	4%	2%	-	>6.0
Control	4%	0%	-	4.3
Control	0%	2% *	-	0

* For explanation see footnote to Table 1.

Synergistic antibacterial activity is demonstrated between benzalkonium chloride and 4-chloro-3,5-dimethylphenol at a ratio of 4:2.

Examples 18-21

A range of formulations were prepared to the following formulae (all percentages are w/v):

TABLE 7

	Example 18	19	20	21
BKC	4%	4%	4%	4%
PCMX	2%	2%	2%	2%
Propan-2-ol	10%	10%	10%	30%
PEG 200*	10%	25%	-	-
PEG 400**	-	-	25%	-
Perfume***	0.25%	0.25%	0.25%	0.25%
Softened water	to 100%	to 100%	to 100%	to 100%

* Polyethylene glycol average molecular weight 200

** Polyethylene glycol average molecular weight 400

*** Felton "Antiseptic Fragrance" 87.1619

The formulations were prepared following the procedure given for Examples 1 to 4.

Examples 22-25

The compositions of Examples 18 to 21 were varied by the addition of 0.75% w/v disodium ethylenediamine tetraacetic acid.

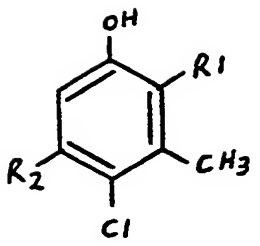
CLAIMS

1. Method for disinfecting bacteriologically contaminated surfaces which comprises the application to said surfaces of a composition comprising a synergistic mixture of (a) a quaternary ammonium compound and (b) a chlorinated methyl substituted phenol, in (c) at least one aqueous mono-hydric alcohol and, optionally, at least one poly-hydric alcohol; wherein the ratio of (a) to (b) is between 4:1 and 1:1.5.

2. A method of disinfection as claimed in Claim 1 wherein the quaternary ammonium compound is of the general formula $[R_1R_2R_3R_4N]^+X^-$ in which each R group is a substituted or unsubstituted alkyl, aryl, alkaryl, aralkyl or alkenyl group or three of the four R groups are combined in a pyridine ring, the sum of the carbon atoms in the four R groups is more than 10, at least one of the R groups has a chain length in the range C_8-C_{18} and X is a small anion.

3. A method of disinfection as claimed in Claim 2 wherein the quaternary ammonium compound is 1-hexadecylpyridinium chloride; benzyldimethyl-2-{2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy}ethylammonium chloride; mixtures of alkyldimethylbenzylammonium chlorides; mixtures of dodecyl-, tetradecyl- and hexadecyl-trimethylammonium bromides; mixtures of alkaryltrimethylammonium chlorides; or mixtures thereof.

4. A method of disinfection as claimed in any preceding Claim wherein the chlorinated methyl substituted phenol is of the general formula (I)



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where R_1 is chlorine or hydrogen, and

R_2 is methyl or hydrogen.

5. A method of disinfection as claimed in Claim 4 wherein the chlorinated methyl substituted phenol is 4-chloro-3-methylphenol, 4-chloro-3,5-dimethylphenol, 2,4-dichloro-3,-5-dimethylphenol or a mixture thereof.

6. A method of disinfection as claimed in any preceding Claim wherein the mono-hydric alcohol is a lower alkyl C_{1-4} alcohol; and the poly-hydric alcohol, when present, is a polyethylene glycol of average molecular weight 200 to 1000, propylene glycol or ethylene glycol.

7. A method of disinfection as claimed in Claim 6 wherein the mono-hydric alcohol is ethanol or propan-2-ol; and the poly-hydric alcohol, when present, is a polyethylene glycol of average molecular weight 200 to 600, or propylene glycol.

8. A method of disinfection as claimed in any preceding Claim wherein the quaternary ammonium compound is at a concentration of 0.02-0.2% w/v and the chlorinated methyl substituted phenol is at a concentration of 0.01-0.15% w/v.

9. A method of disinfection as claimed in Claim 8 whereby

the composition also contains upto 0.10% w/v of a sodium or potassium salt of ethylenediamine tetraacetic acid.

10. A composition for use in the method as claimed in any one of Claims 1 to 9 comprising a synergistic mixture of (a) a quaternary ammonium compound and (b) a chlorinated methyl substitued phenol, in (c) at least one aqueous mono-hydric alcohol and, optionally, at least one poly-hydric alcohol; wherein the ratio of (a) to (b) is between 4:1 to 1:1.5.

11. A concentrated solution for dilution with water to afford a composition as claimed in Claim 10.

12. A concentrated composition for use in disinfection as described in any one of Examples 1 to 25.

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